Normal Bacterial Floras in Intestinal Tract of Ring-Necked Pheasant[1]

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Abstract The normal bacterial floras in intestinal tract of ring-necked pheasant were investigated. Eight age groups were chosen. Samples of intestines were diluted in 10 fold series and incubated on different selective media. After incubation, aimed bacterial colonies were counted then the number of CFU/g of gut inclusions was evaluated. The data were analyzed in statistics. The physiological values of eight main normal bacterial floras were obtained. The eubiosis of normal bacterial floras in intestinal tract of ring-necked pheasant was established from its age of 10 days old to 30 days old. The dominant bacterial floras were bifidobacterium, lactobacillus and bacteroideceae.
key words: Normal bacterial flora, Ring-necked pheasant, Intestinal tract

Introduction

Many micro-floras can be found on skin, organs and tissues, which are opened to the outside. These micro-floras and hosts coexist depend on each other and form a co-mplicated micro-ecological system; in which the normal bacterial floras play an important role. Many reports showed that normal bacterial floras play an important role in the development of immune system. They can resist the invading and of alien bacteria and enhance the ability to resist infection (Kan Bai 1980, and Li Meitong 1991). The normal bacterial floras are significant in maintaining balance of nitrogen in body, in synthesis of vitamin, absorption of lipid and mineral and utility of carbohydrate (March,B.E.1979). It also has a significant influence on growth, development and senility (Kan bai1980).

The research on normal bacterial floras of intestinal tract has been advanced recently and some important results have been achieved in preventing from infection and promoting ability of reproduction(Wan Guoshun 1992).

Micro-ecological products, based on regulating remedy of normal bacterial flora, make it possible to solve these problems which are aroused by medicine-resistant bacterial, re-infection and medical residence resulted from the use of antibiotic. Although some advances have been achieved in research on normal bacterial flora of ring-necked pheasant in intestinal tract. The further basic and systematic researches are still needed.

It was carried out with the theory and method of micro-ecology to make qualitative and quantitative analysis on normal bacterial floras. Then we can obtain physiological values of some normal bacterial flora.

Materials and Methods

Experimental materials

Ring-necked pheasants, used in this study, were obtained from Songpu Town of Daowai District, and Ping Shan Experiment Base of Wildlife Research Institute of Heilongjiang Province.

Sample collection, dilution and dripping

Obtaining of sample Ring-necked pheasants treaded with 3% soap cresol solution were killed immediately. These ring-necked pheasants were chosen with the age of 1, 2, 5, 10, 20, 30, 60, 120 days old respectively. Five ring-necked pheasants were killed in each age group, 0.1-gram dung sample was obtained from the middle part of duodenum, jejunum, cecum and rectum.

Sample dilution Diluting liquid was composed of Na_2HPO_4 (6g), KH_2PO_4 (4.5g), L-cys (0.5g), agar (1g), tween (800.5g) and the total volume is 1000ml. After sterilized, the dung samples were diluted in 1o fold series with this liquid.

Dripping method Dripping planting was conducted on every selectively culture medium from the highest diluting liquid. Three different diluting liquids were dropped on a plate, repeated 3 times. Different diluting times were needed to different samples.

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Culture medium and cultural method

Culture medium BS (bifidobacterium culture medium) was used to selectively culture bifidobacterium. LBS (lactobacillus culture medium) was used to selectively culture lactobacillus. PMS (peptococcus culture medium) was used to selectively culture peptococcus. NBGT (bacteroides culture medium) was used to se-lectively culture bacteroides. ES (eubacterium culture medi-um) was used to selectively culture eubacterium. CD (clostridium culture medium) was used to selectively culture clostridium. TATAC (streptococcus culture medium) was used to selectively culture streptococcus. EMB (escherichia coli culture medium) was used to selectively culture escherichia coli respectively.

Culture method Aerobe and facultative anaerobe were cultured in incubator. Anaerobe were culture in anaerobic jar, cultural gases were 8% N_2, 10% CO_2, 10% H_2. Granulated palladium was used to catalyze consumption of oxygen, desiccation was used to absorb excessive water, active carbon was used to absorb H_2S, as well as mic-roharmful gases. Culture time was 72h. Temperature was 35 °C

Counting and identifying of bacteria Counting was carried out based on the countable principal, then average number was calculated, thus, the number of bacteria contained in per gram dung could be obtained, the following was:

The number of bacteria in per gram dung = average of bacterialcolony × the number of dropping × diluting times.

Denitrifying of bacteria While counting bacteria, bacteria from colonies, which were cultured on the selective culture medium, were stained by Gram's method and examined under the microscope. If necessary, some biochemistry tests were used to help further identify.

Results

The logarithmic values of the number of bacteria in per gram dung was calculated the average of logarithmic values from five birds, and their standard deviation was calculated. Now, the scope of average plus or minus the standard deviation was obtained. Table1 shows the number of anaerobe in the different regions of intestinal track of ring-necked pheasants whose age was 1, 2, 5, 10, 20, 30, 60, 120 days old. Table 2 shows the number of aerobe in the different regions of intestinal track of ring-necked pheasants whose age is aforementioned.

From Table 1, we know bifidobacterium could be examined in duodenum 2 days after birth, in jejunum 5 days, in cecum and rectum 10 days, lactobacillus and clostridium appeared in different regions of intestinal track 1 day after birth, bacteroides. Peptococcus and eubacterium began to appear from 5 days to 10 days after birth, the constant number of bacteria could be formed. The multiplication period of these six bacteria was 10 to 30 days after birth. After 30 days after birth, the dominant bacteria in duodenum and jejunum were bifidiobacterium lact-obacillus, peptococcus. In cecum and rectum were bifidobacterium, lactobacillus, peptococcus and bacteroides.

From Table 2, we knew escherichia coli, and streptococcus could be examined in different region of intestinal tract 1 day after birth. The logarithmic of escherichia coli reached to highest, 10 to 20 days after birth, and began to decline gradually. After 30 days, the number was constant ($P>0.05$). Streptococcus grew fast in intestinal tract; the logarithmic of the number of streptococcus began to approach balance after 20 to 30 days after birth, and became the most dominant bacteria in duodenum.

Discussion and Conclusions

The reports on normal bacterial flora of the intestines of ring-necked pheasant have not been found today, the only evidence that we could used to refer and compare was from results about chicken. Yang Jingyun (1991) reported that the physiological values of normal bacterial flora of chicken's cecum were bifidobacterium (7.15±0.15), lactobacillus (7.00±0.11), peptococuus (5.91±0.22), streptoc-occus (6.10±0.16), escherichia coli (5.10±0.24). It was similar to our experimental results. The fact showed that the physiological values of normal bacterial flora in ring-necked pheasant intestinal tract were same as that in chicken. This result provides scientific basis for the study of intestinal tract bacterial disease of ring-necked pheasant or micro-organism products for epidemic disease prevention.

Anaerobes are dominant normal bacterial flora in ring-necked pheasant intestinal tract. The state of normal bacterial flora of ring-necked pheasant intestinal tracts is established from its age of 10 days old to 30 days old. Destroying the state, the dysbacteriosis would be happen. Ring-necked pheasants are more easily infected by bacterial in this period. The fact also has been proved by clinical experience.

Physiological function and procession, kinds of feed, methods of raising can affect the normal bacterial flora physiological values of ring-necked pheasant intestinal tract. This is a common problem. For example, affected by surrounding factors, the stomach of chicken secretes can increase intensely and intestines wriggles can stop. Escherichia coli would breed sharply in duodenum and jejunum that leads to diarrhea. Bacterial flora changes with feed changing. We should consider these factors when detecting and analyzing pheasant intestinal tract normal bacterial flora.

Table 1. The number of normal anaerobe floras of ring-necked pheasant in intestinal tract (lg *N*)

Intesties	Bacteria	1 days old	2 days old	5 days old	10 days old	20 days old	30 days old	60 days old	120 days old
duodenum	bifidobacterium	0	1.44 ± 0.03	2.56 ± 0.26	4.53 ± 0.18	6.15 ± 0.13	6.28 ± 0.18	6.75 ± 0.09	6.35 ± 0.12
	lactobacillus	2.54 ± 0.02	3.74 ± 0.12	6.33 ± 0.18	6.45 ± 0.03	6.43 ± 0.12	7.01 ± 0.18	6.35 ± 0.12	6.22 ± 0.09
	bacteroides	0	0	0	3.12 ± .08	6.13 ± 0.12	7.03 ± 0.05	7.12 ± 0.14	7.13 ± 0.06
	peptococcus	0	0	0	1.02 ± 0.11	1.10 ± 0.12	0	0	0
	eubacterium	0	0	0	0	2.85 ± 0.01	3.23 ± 0.06	3.11 ± 0.07	3.45 ± 0.18
	clostridium	3.12 ± 0.10	3.43 ± 0.09	3.45 ± 0.18	3.47 ± 0.07	3.99 ± 0.08	4.01 ± 0.01	4.12 ± 0.01	4.15 ± 0.07
jejunum	bifidobacterium	0	0	1.32 ± 0.12	2.89 ± 0.13	6.12 ± 0.14	6.58 ± 0.25	6.97 ± 0.04	6.32 ± 0.18
	lactobacillus	2.42 ± 0.10	3.67 ± 0.01	6.37 ± 0.11	6.43 ± 0.12	6.52 ± 0.04	6.88 ± 0.13	6.12 ± 0.01	6.39 ± 0.08
	bacteroides	0	0	0	3.15 ± 0.04	6.78 ± 0.11	6.56 ± 0.04	6.72 ± 0.03	6.87 ± 0.14
	peptococcus	0	0	0	0	2.10 ± 0.10	2.33 ± 0.11	2.11 ± 0.10	2.45 ± 0.18
	eubacterium	0	0	0	0	3.10 ± 0.11	3.14 ± 0.10	3.10 ± 0.12	3.11 ± 0.07
	clostridium	3.14 ± 0.07	3.12 ± 0.18	3.10 ± 0.22	3.17 ± 0.22	3.80 ± 0.17	4.10 ± 0.15	4.10 ± 0.15	4.11 ± 0.04
cecum	bifidobacterium	0	0	0	2.31 ± 0.05	4.32 ± 0.11	7.35 ± 0.12	7.67 ± 0.18	7.76 ± 0.03
	lactobacillus	2.51 ± 0.11	3.42 ± 0.05	6.43 ± 0.02	6.31 ± 0.07	6.35 ± 0.01	6.47 ± 0.21	6.74 ± 0.41	6.89 ± 0.05
	bacteroides	0	0	0	3.14 ± 0.05	7.01 ± 0.13	7.14 ± 0.22	7.31 ± 0.41	7.15 ± 0.08
	peptococcus	0	0	0	2.23 ± 0.11	5.67 ± 0.15	7.11 ± 0.17	7.22 ± 0.34	7.20 ± 0.04
	eubacterium	0	0	0	2.89 ± 0.17	3.10 ± 0.10	3.12 ± 0.05	3.41 ± 0.12	3.12 ± 0.07
	clostridium	3.01 ± 0.08	3.46 ± 0.27	3.59 ± 0.08	3.15 ± 0.17	4.01 ± 0.19	3.88 ± 0.17	3.86 ± 0.11	3.86 ± 0.20
rectum	bifidobacterium	o	0	0	2.43 ± 0.02	4.31 ± 0.19	7.36 ± 0.19	7.38 ± 0.25	7.20 ± 0.17
	lactobacillus	0	2.42 ± 0.05	4.32 ± 0.11	6.12 ± 0.07	6.43 ± 0.12	7.01 ± 0.12	6.80 ± 0.07	6.90 ± 0.01
	bacteroides	0	0	0	3.15 ± 0.05	6.89 ± 0.11	6.60 ± 0.04	6.72 ± 0.03	7.10 ± 0.12
	peptococcus	0	0	0	0	3.14 ± 0.12	6.73 ± 0.18	6.89 ± 0.15	7.10 ± 0.13
	eubacterium	0	0	0	2.10 ± 0.16	2.89 ± 0.17	3.10 ± 0.07	3.45 ± 0.04	3.43 ± 0.09
	clostridium	2.15 ± 0.10	2.10 ± 0.04	3.41 ± 0.12	3.48 ± 0.19	3.41 ± 0.17	3.41 ± 0.17	3.40 ± 0.04	3.10 ± 0.12

Table 2. The number of normal aerobe floras of ring-necked pheasant in intestinal tract (lg*N*)

Intesties	Bacteria	1 days old	2 days old	5 days old	10 days old	20 days old	30 days old	60 days old	120 days old
duodenum	escherichia	3.15 ± 0.12	4.13 ± 0.01	5.67 ± 0.18	5.89 ± 0.11	6.01 ± 0.08	6.13 ± 0.13	4.12 ± 0.31	4.13 ± 0.14
	streptococcus	4.31 ± 0.15	5.12 ± 0.09	6.10 ± 0.18	6.10 ± 0.18	6.90 ± 0.31	7.25 ± 0.19	7.33 ± 0.11	7.14 ± 0.02
jejunum	escherichia	2.85 ± 0.02	3.44 ± 0.15	6.10 ± 0.03	6.11 ± 0.25	6.23 ± 0.17	5.88 ± 0.10	4.05 ± 0.09	4.18 ± 0.17
	streptococcus	3.11 ± 0.15	3.43 ± 0.02	3.58 ± 0.08	4.31 ± 0.17	5.55 ± 0.32	6.10 ± 0.01	5.89 ± 0.30	5.62 ± 0.14
cecum	escherichia	3.17 ± 0.01	3.48 ± 0.02	5.39 ± 0.14	6.89 ± 0.13	7.01 ± 0.06	5.89 ± 0.19	4.52 ± 0.31	4.11 ± 0.03
	streptococcus	2.10 ± 0.31	2.45 ± 0.01	3.10 ± 0.21	3.45 ± 0.27	4.41 ± 0.13	4.44 ± 0.08	4.80 ± 0.07	4.34 ± 0.01
rectum	escherichia	21.0 ± 0.43	3.15 ± 0.10	5.10 ± 0.31	6.18 ± 0.18	6.72 ± 0.21	5.10 ± 0.13	4.10 ± 0.31	4.55 ± 0.22
	streptococcus	1.90 ± 0.31	2.08 ± 0.31	2.45 ± 0.31	3.41 ± 0.07	4.10 ± 0.25	4.67 ± 0.10	4.67 ± 0.10	4.10 ± 0.40

The experiment was carried out with the method of diluting, dripping and planting. It was conducted in qualitative and quantitative analysis on eight kinds of bacteria from contents of intestinal tract in different days after birth from four different regions. The results were calculated in statistics and physiological values of eight main normal bacterial floras were obtained. The eubiosis of intestinal tract normal bacterial flora of ring-necked pheasant was established from 10 days old to 30 days old. The dominant bacterial floras were as follows: Duodenum was streptococcus, bacteroideceae. Jejunum was bifidobacterium, lactobacillus, and bacteroideceae. Cecum and rectum were bifidobacterium, bacteroideceae, and peptococcus.

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